In Vitro Growth of CD4+ Peripheral Blood T Cells in Serum-Free Medium

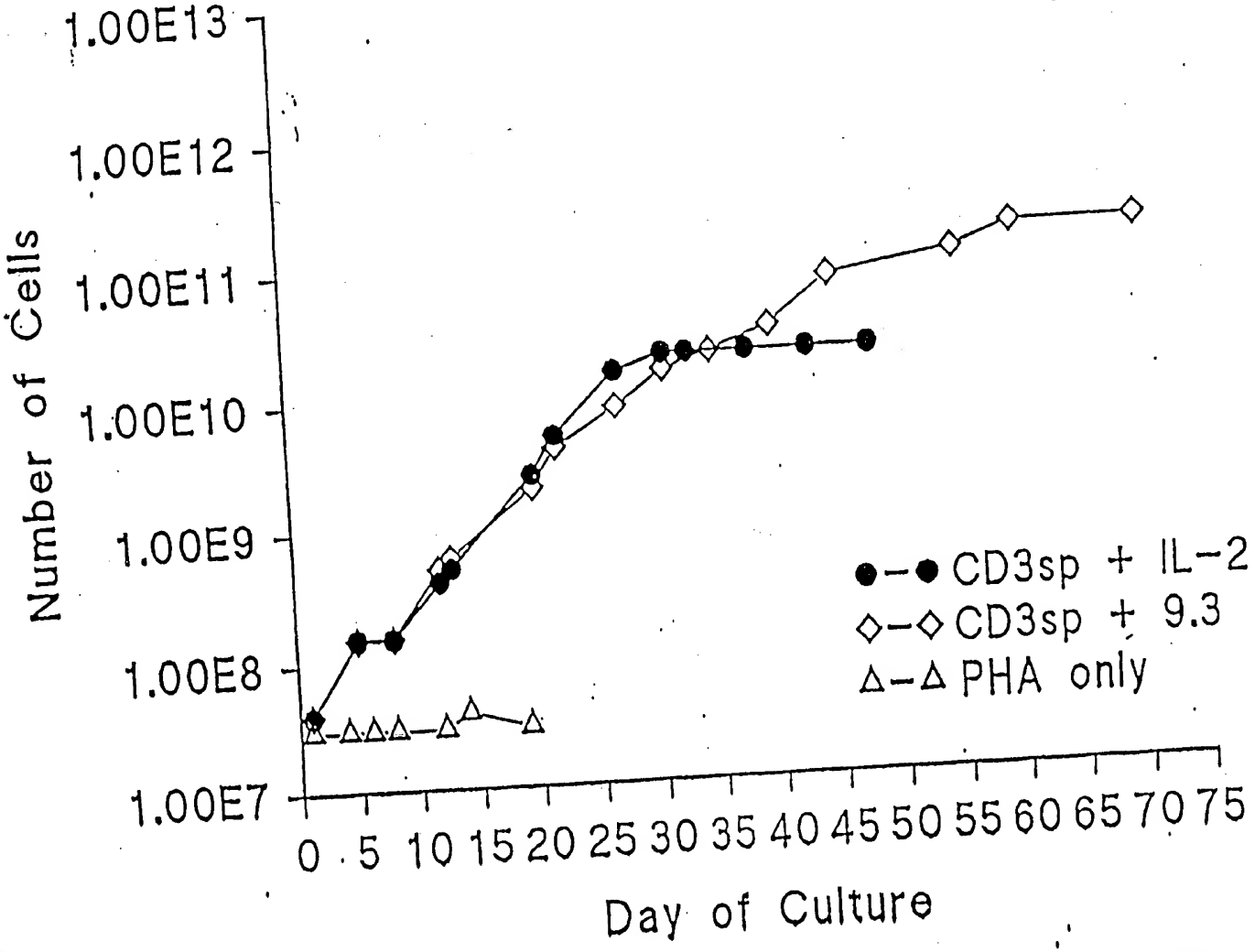


FIG. 1

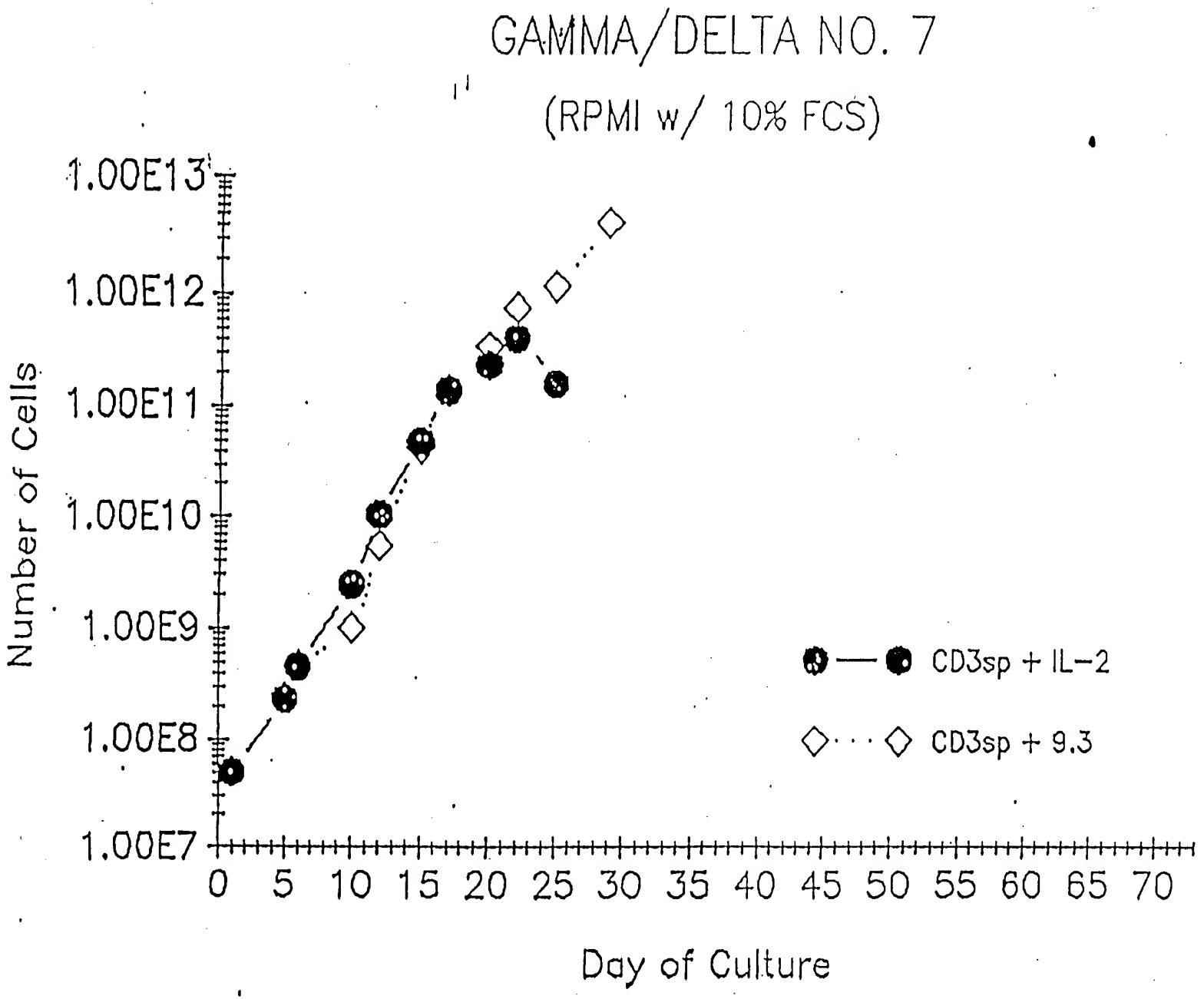

GAMMA/DELTA NO. 7
(RPMI w/ 10% FCS)
Number of Cells
1.00E13
1.00E12
1.00E11
1.00E10
1.00E9
1.00E8
1.00E7
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
Day of Culture
CD3sp + IL-2
CD3sp + 9.3

FIG. 2

LT #13: GROWTH CURVES

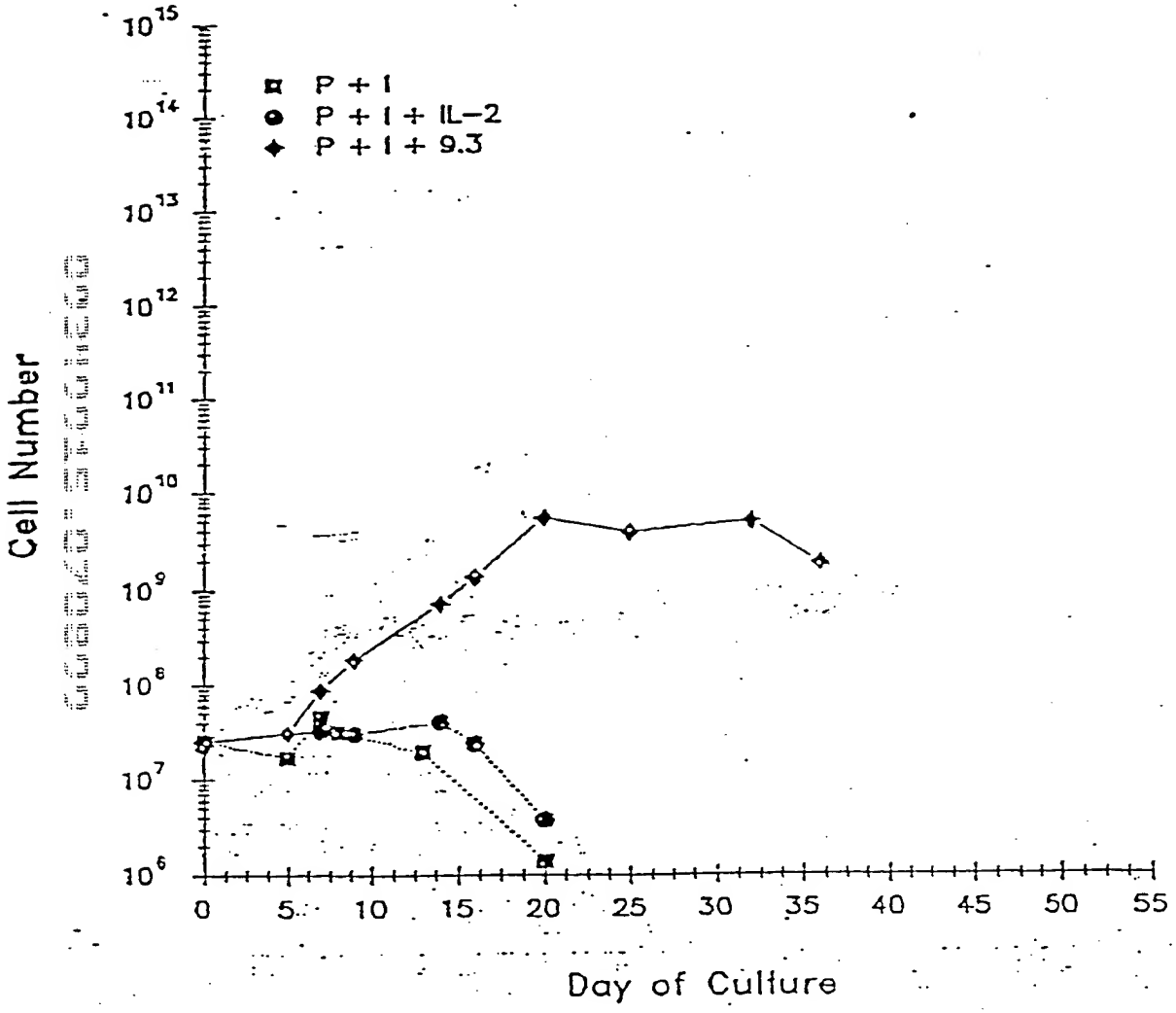


FIG. 3

Selective Outgrowth of $CD4^+$ T Cells During CD28 Stimulated T Cell Expansion

PBMN → α-CD3 + IL-2 → Phenotype d.16

PBMN → α-CD3 + α–CD28 → Phenotype d.16

Culture	Day 0		Day 16	
	CD4	CD8	CD4	CD8
	30.9	13.9		
CD3 + IL-2			8.0	84.5
CD3 + CD28			44 6	52.5

FIG. 4

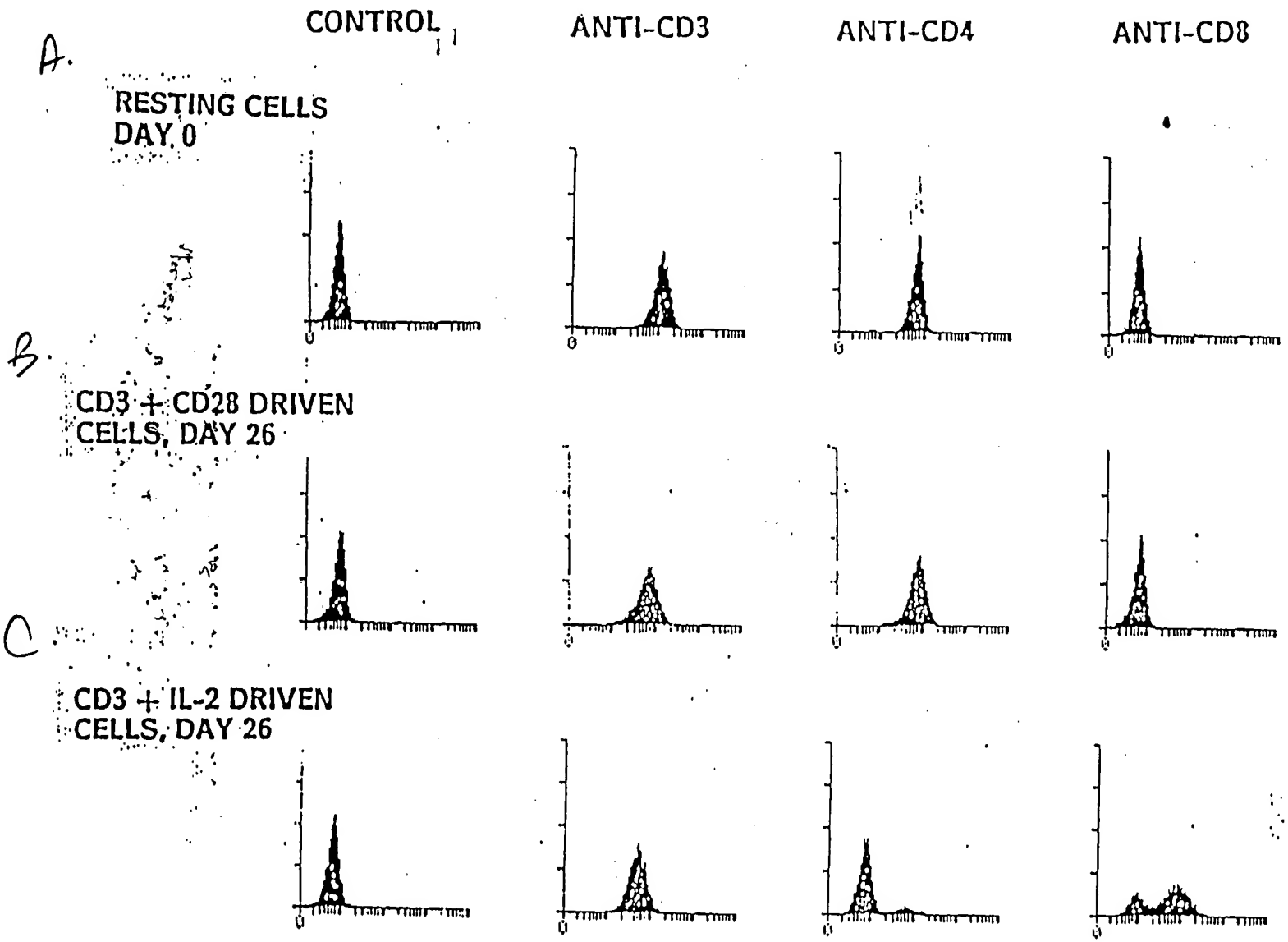

CONTROL
ANTI-CD3
ANTI-CD4
ANTI-CD8
A.
RESTING CELLS
DAY 0
B.
CD3 + CD28 DRIVEN
CELLS, DAY 26
C
CD3 + IL-2 DRIVEN
CELLS, DAY 26

FIG. 5

mAb EX5.3D10

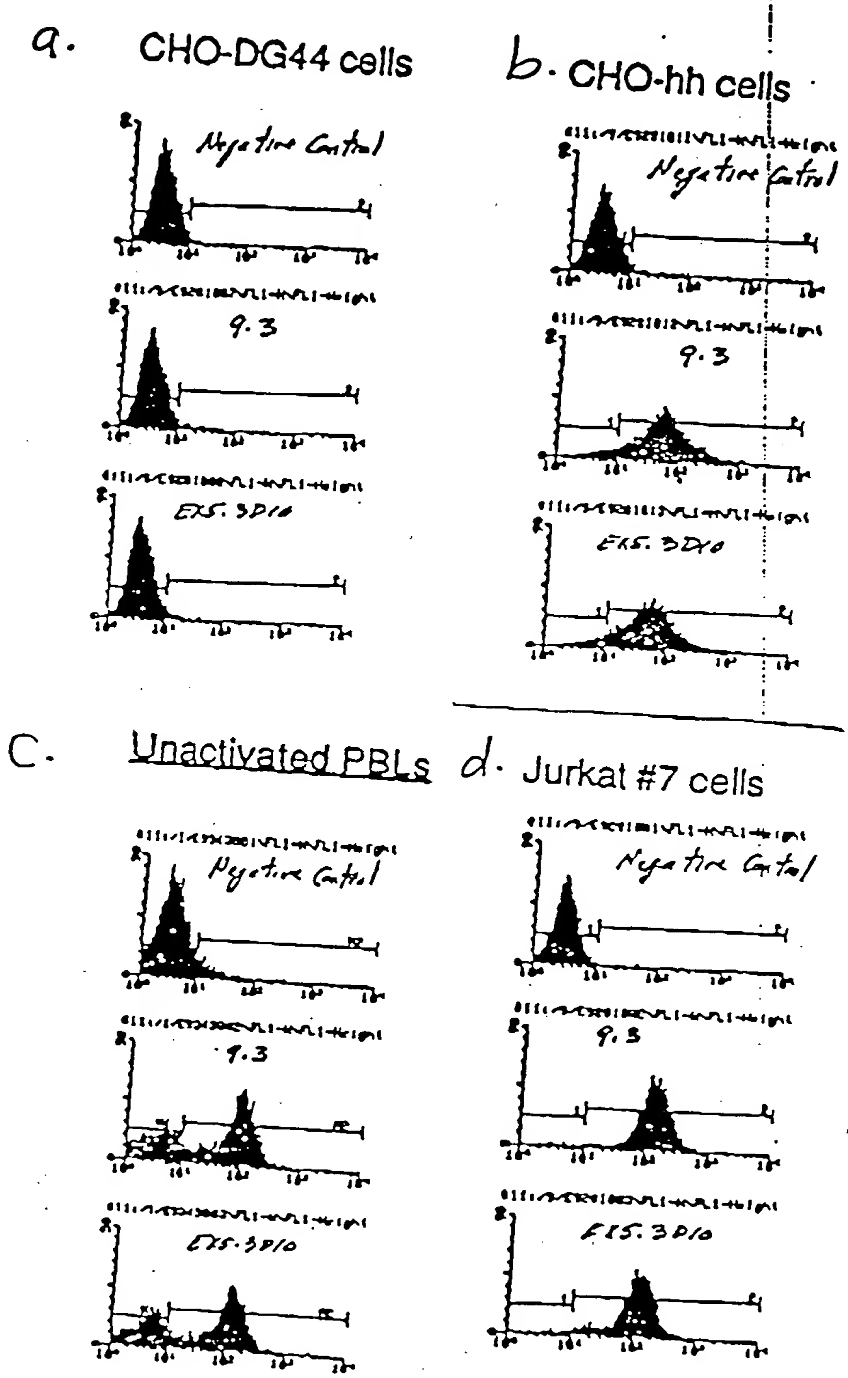
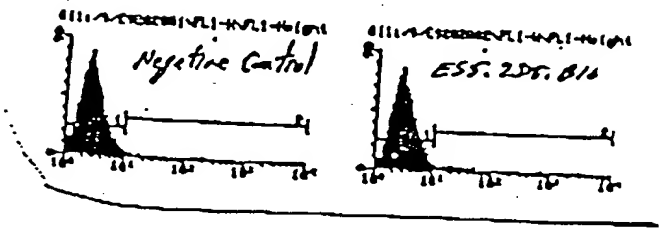


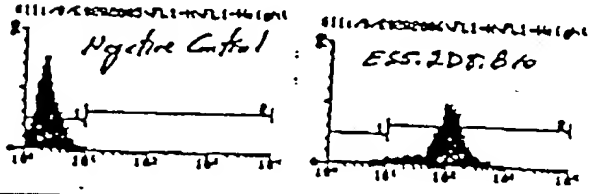
FIG. 6

mAb ES5.2D8

a. CHO-DG44 cells



b. CHO-105A cells



hPBLs:

c. Unactivated

d. PMA Activated

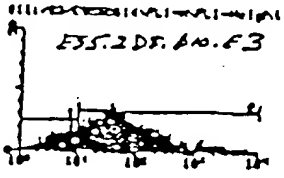


FIG. 7

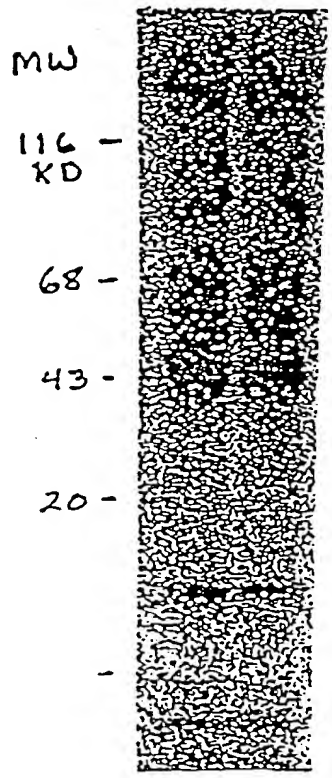


FIG. 8

Use of Cell Sizing to Propagate $CD4^+$ T Cells

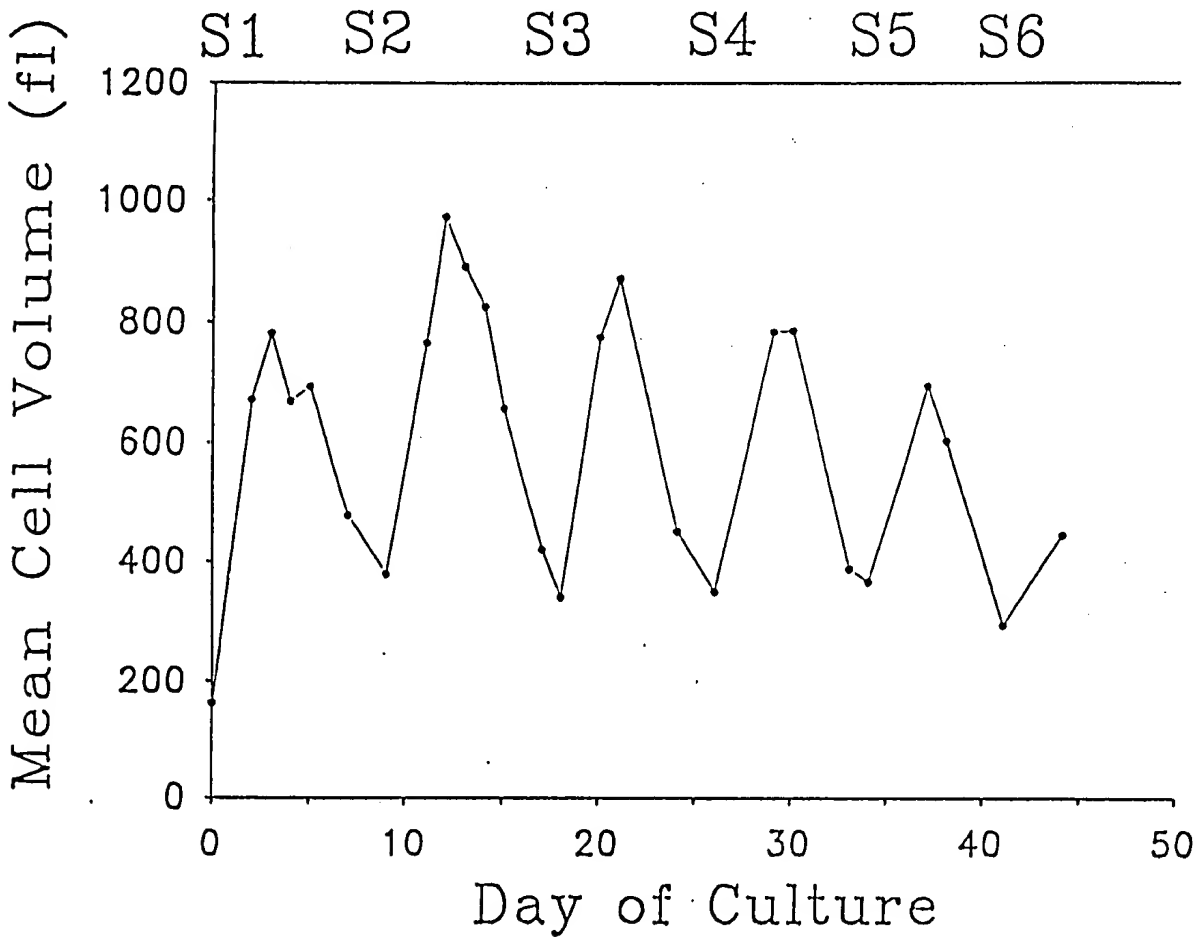
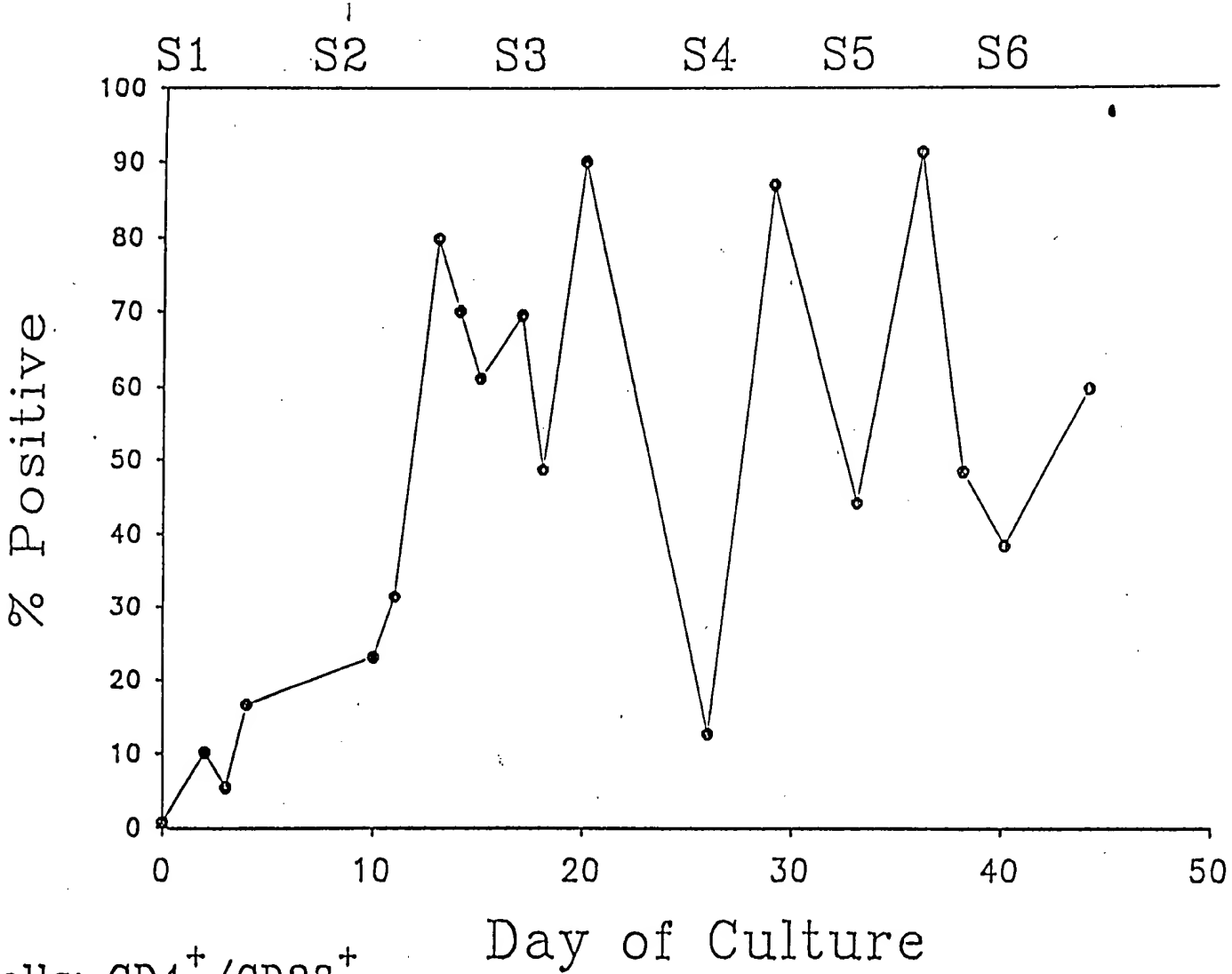


FIG. 9

Cells: $CD28^+/CD4^+$
Stimulation: CD3sp + CD28

Cyclic Expression of B7 on $CD4^+$ T Cells



Cells: $CD4^+/CD28^+$

Stimulation: CD3sp + CD28

FIG. 10

Cytokine Accumulation in Culture Supernatants

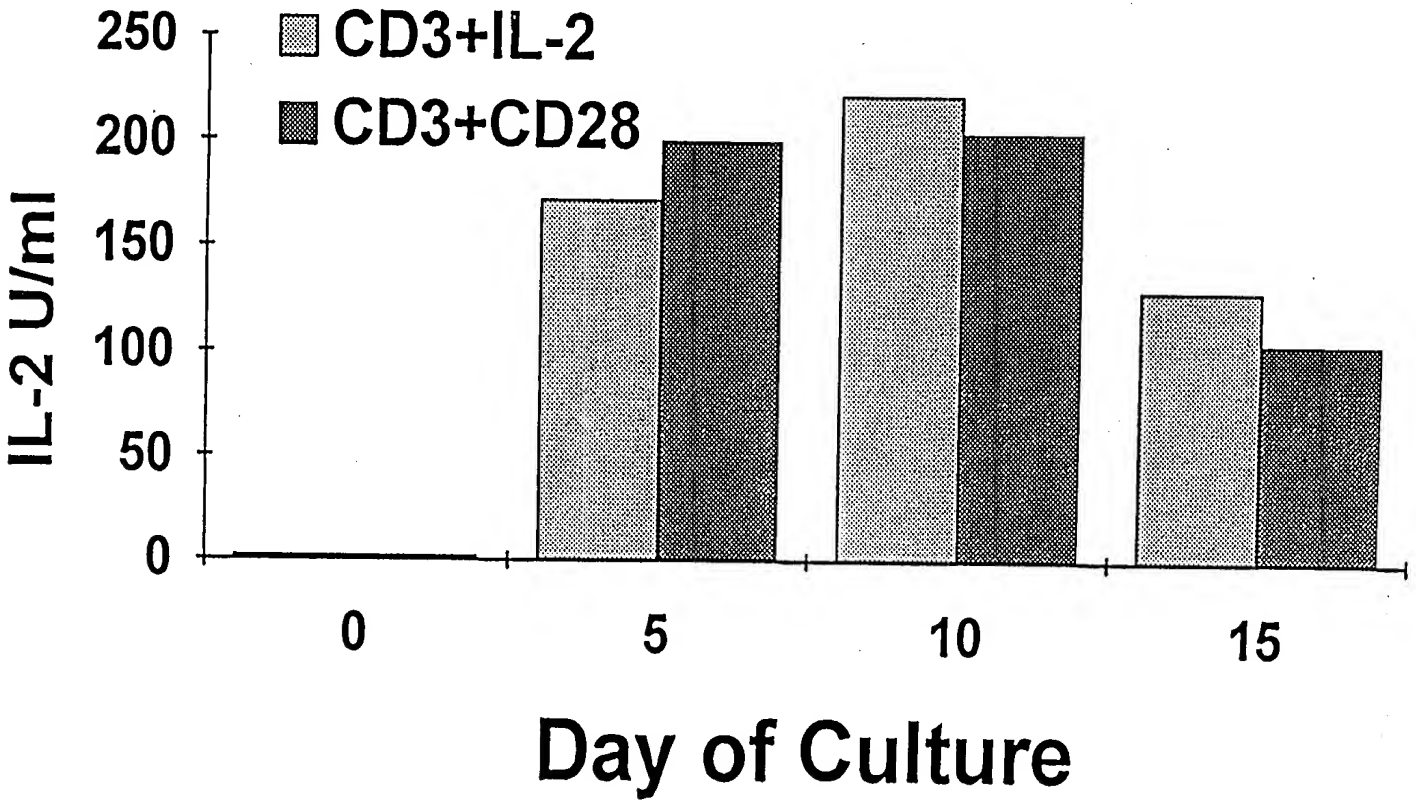


FIG. 11

Cytokine Accumulation in Culture Supernatants

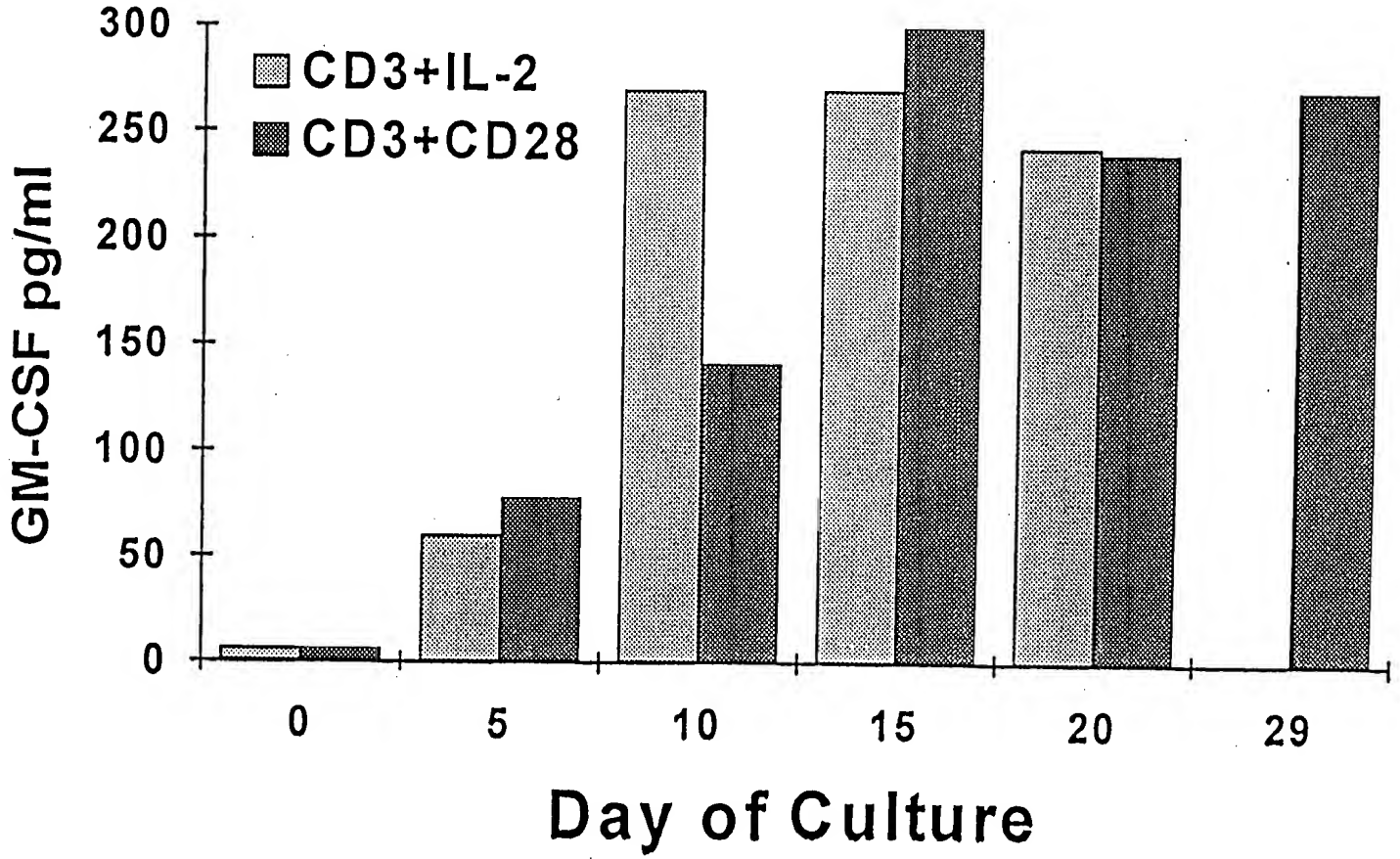


FIG. 12

Cytokine Accumulation in Culture Supernatants

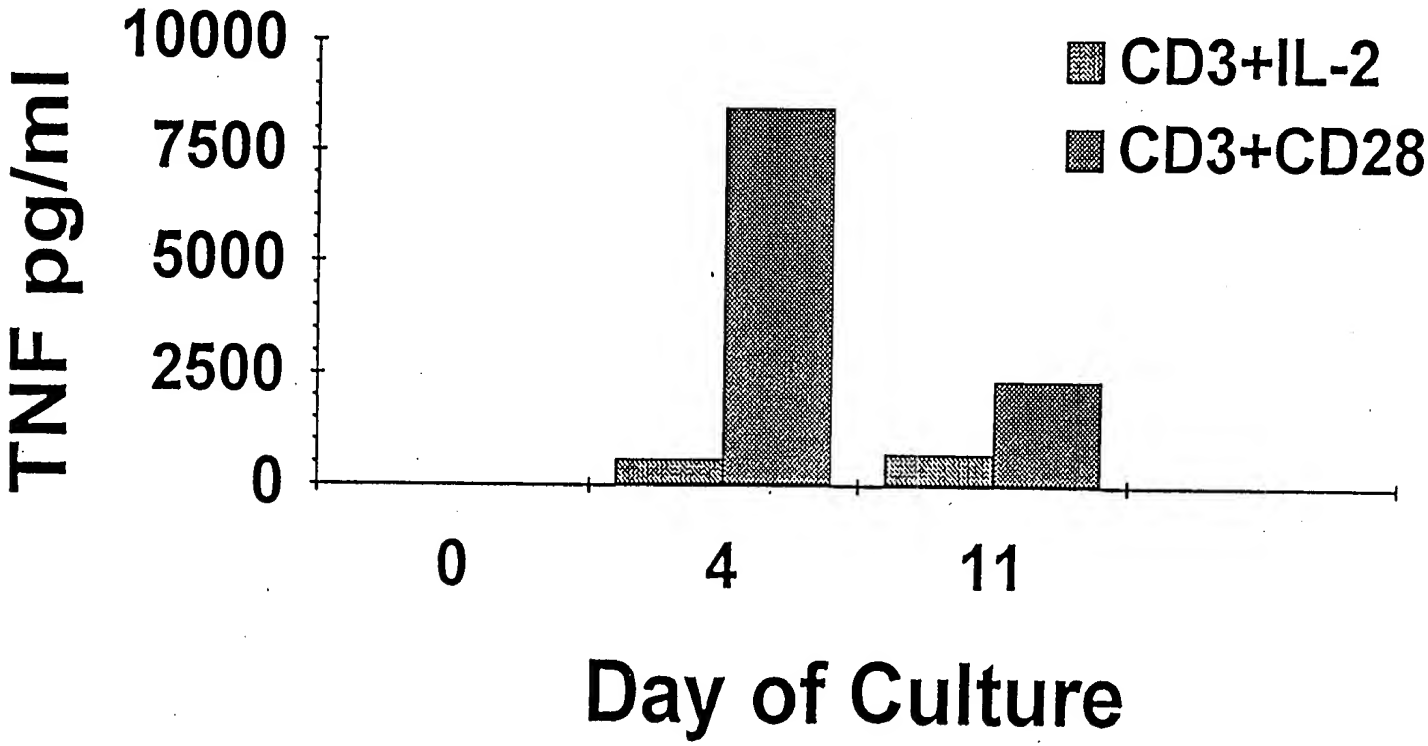


FIG. 13

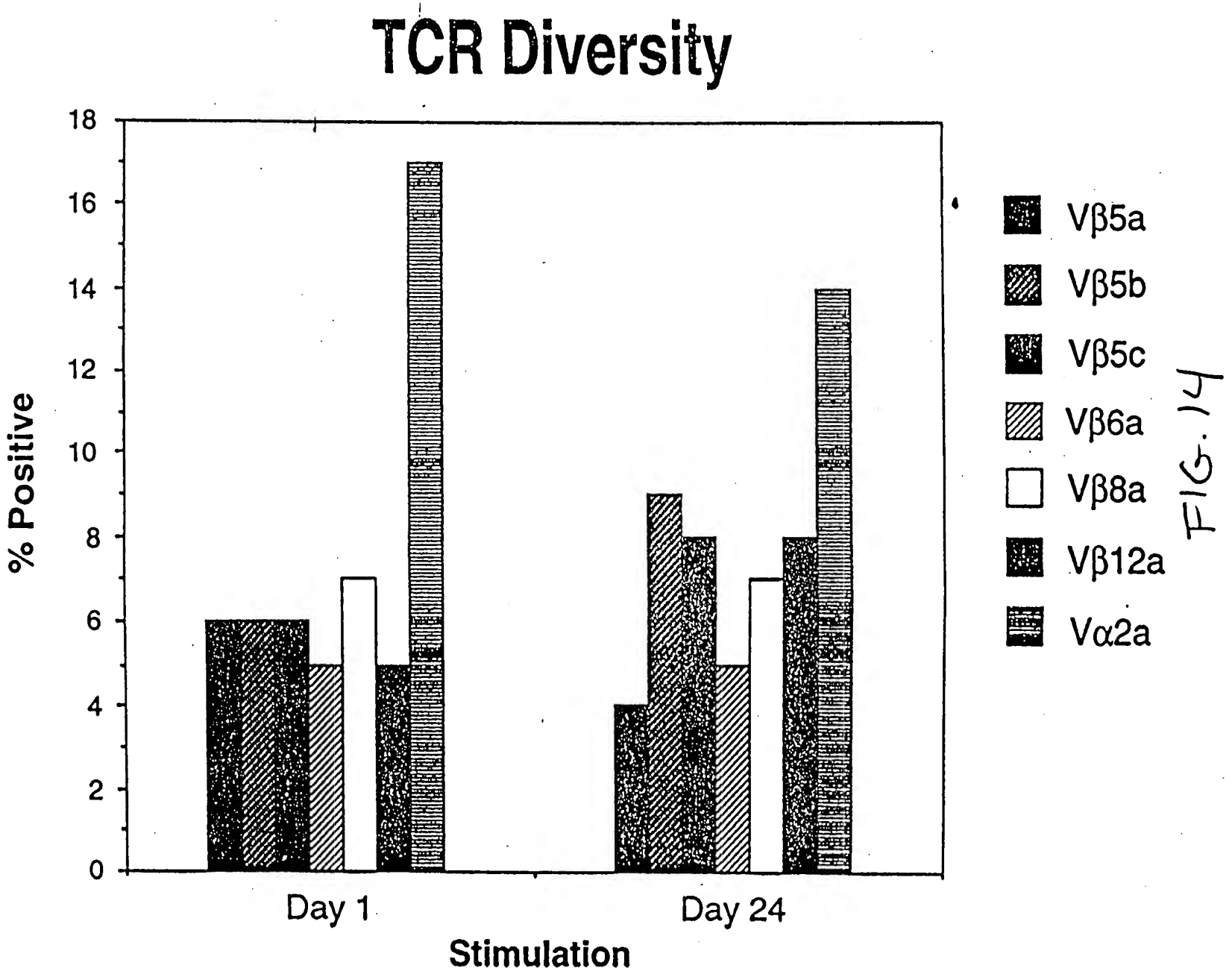

TCR Diversity
% Positive
18
16
14
12
10
8
6
4
2
0
Day 1
Day 24
Stimulation
Vβ5a
Vβ5b
Vβ5c
Vβ6a
Vβ8a
Vβ12a
Vα2a

FIG. 14

(HIV-) Cell Surface Staining

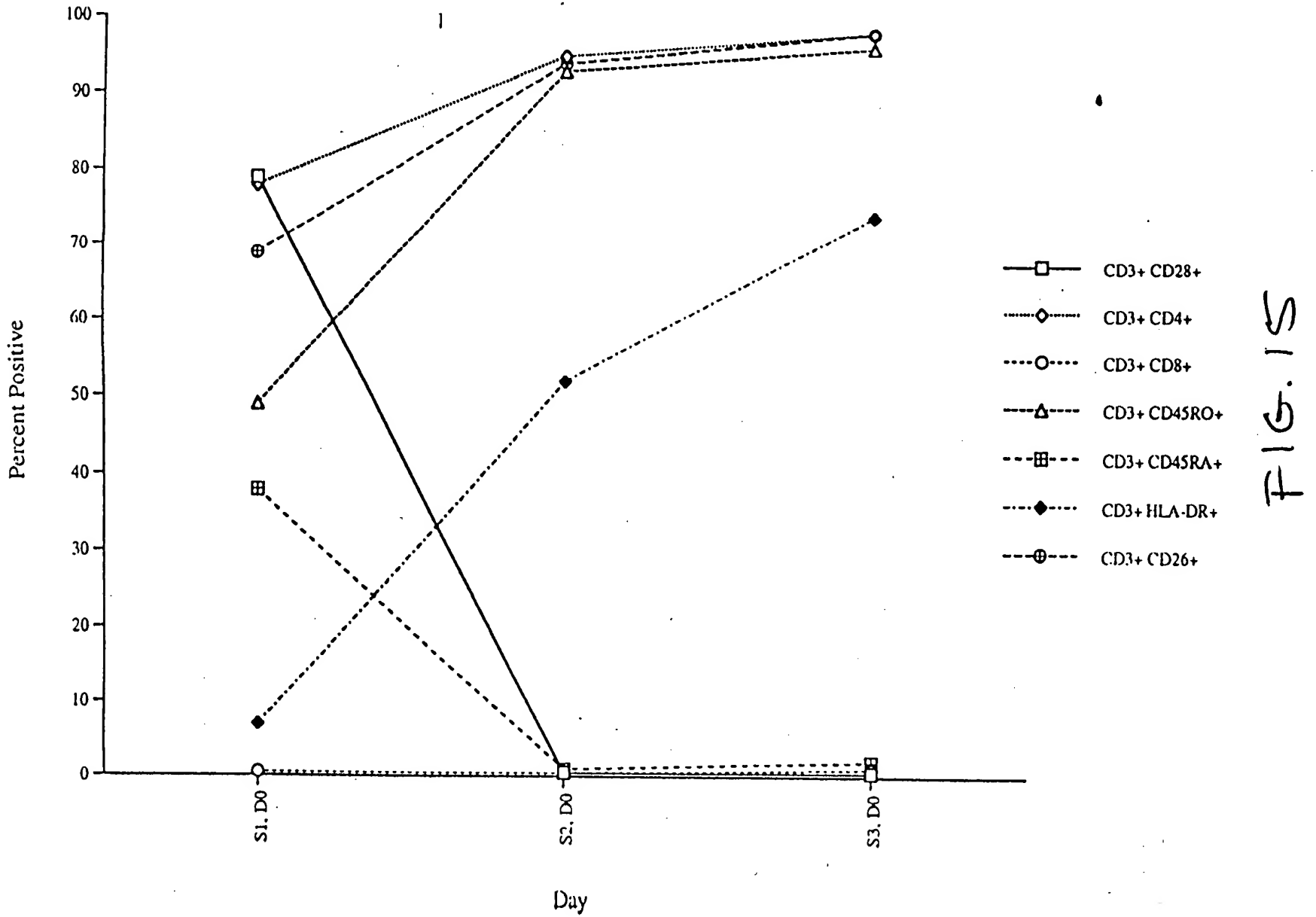


Fig 17

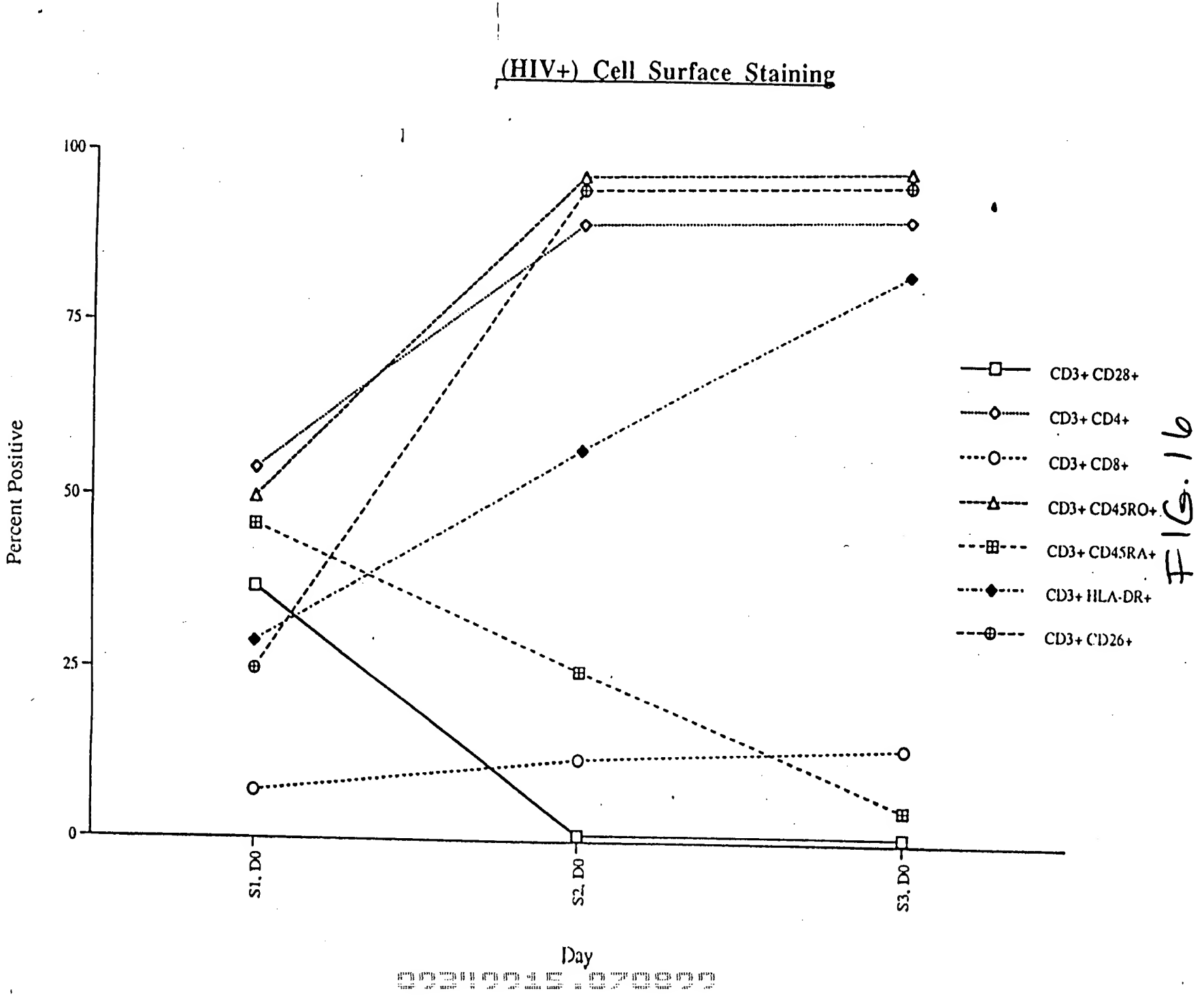
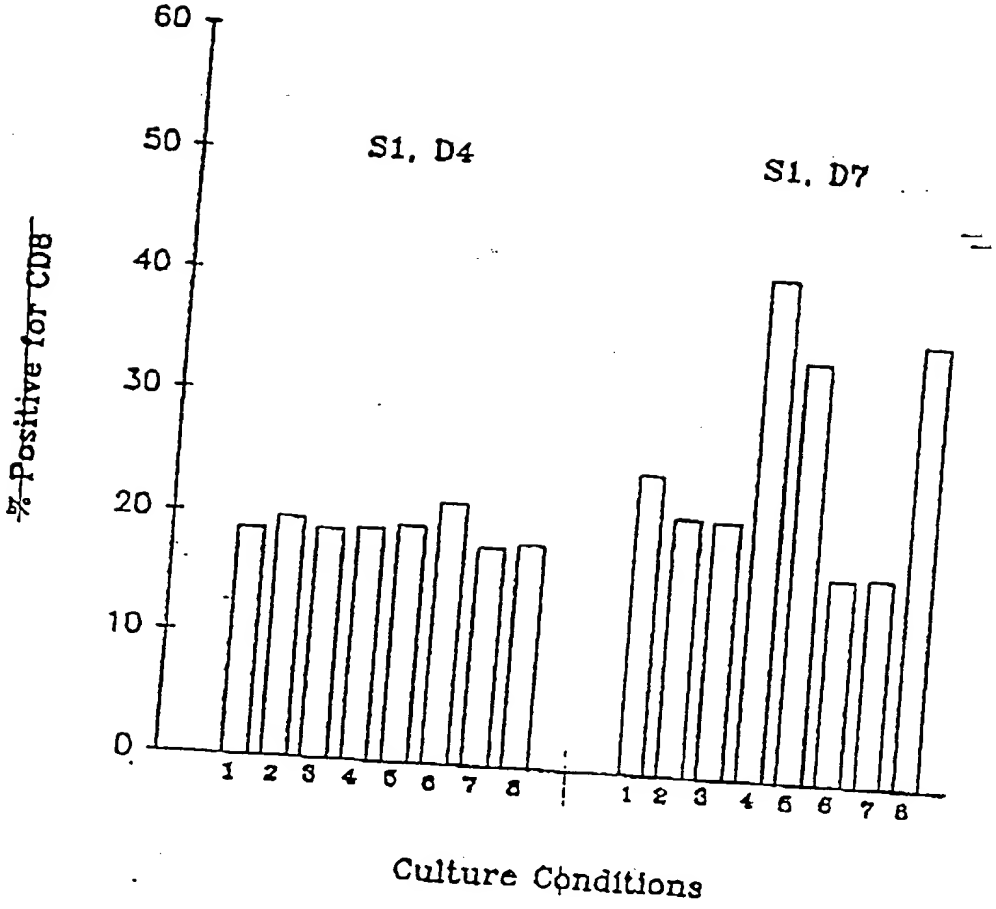

(HIV+) Cell Surface Staining
Percent Positive
100
75
50
25
0
S1, D0
S2, D0
S3, D0
Day
CD3+ CD28+
CD3+ CD4+
CD3+ CD8+
CD3+ CD45RO+
CD3+ CD45RA+
CD3+ HLA-DR+
CD3+ CD26+

FIG. 16



#1: G19-4sp + IL-2
#2: G19-4sp + 9.3
#3: G19-4sp + EX5.3D10
#4: G19-4sp + ES4.2D8
#5: G19-4sp + 7G11
#6: G19-4sp + 9.3 + ES4.2D8
#7: G19-4sp + 9.3 + 7G11
#8: G19-4sp + ES4 + 7G11

FIG. 17

MOP cells transfected with plasmid encoding:

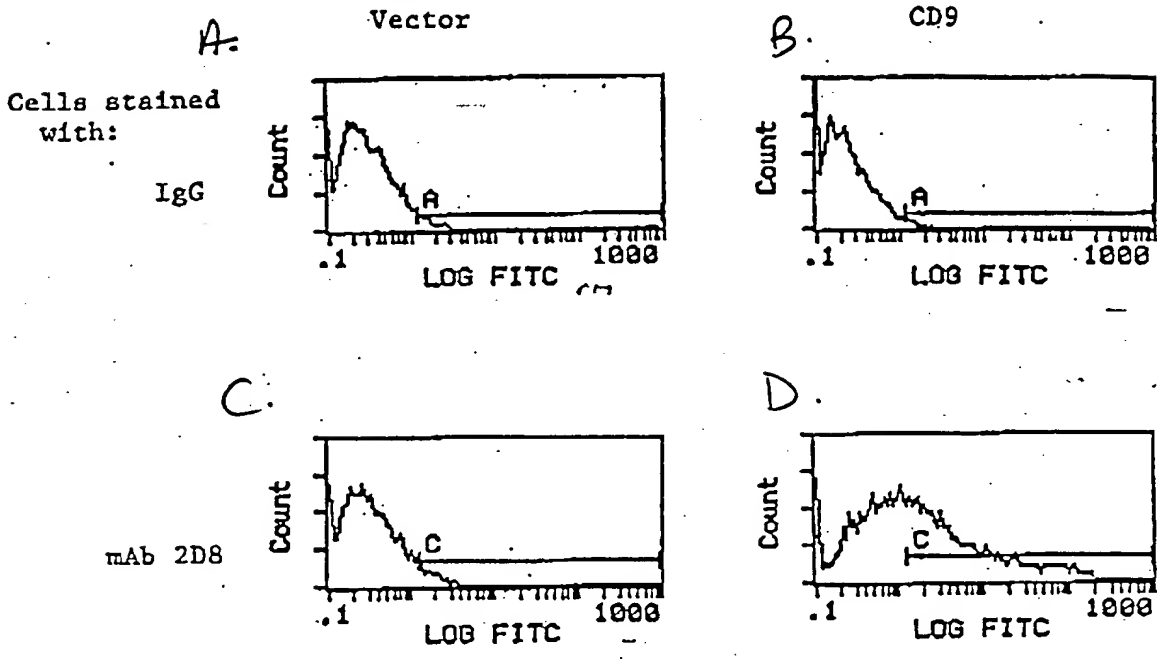


FIG. 18

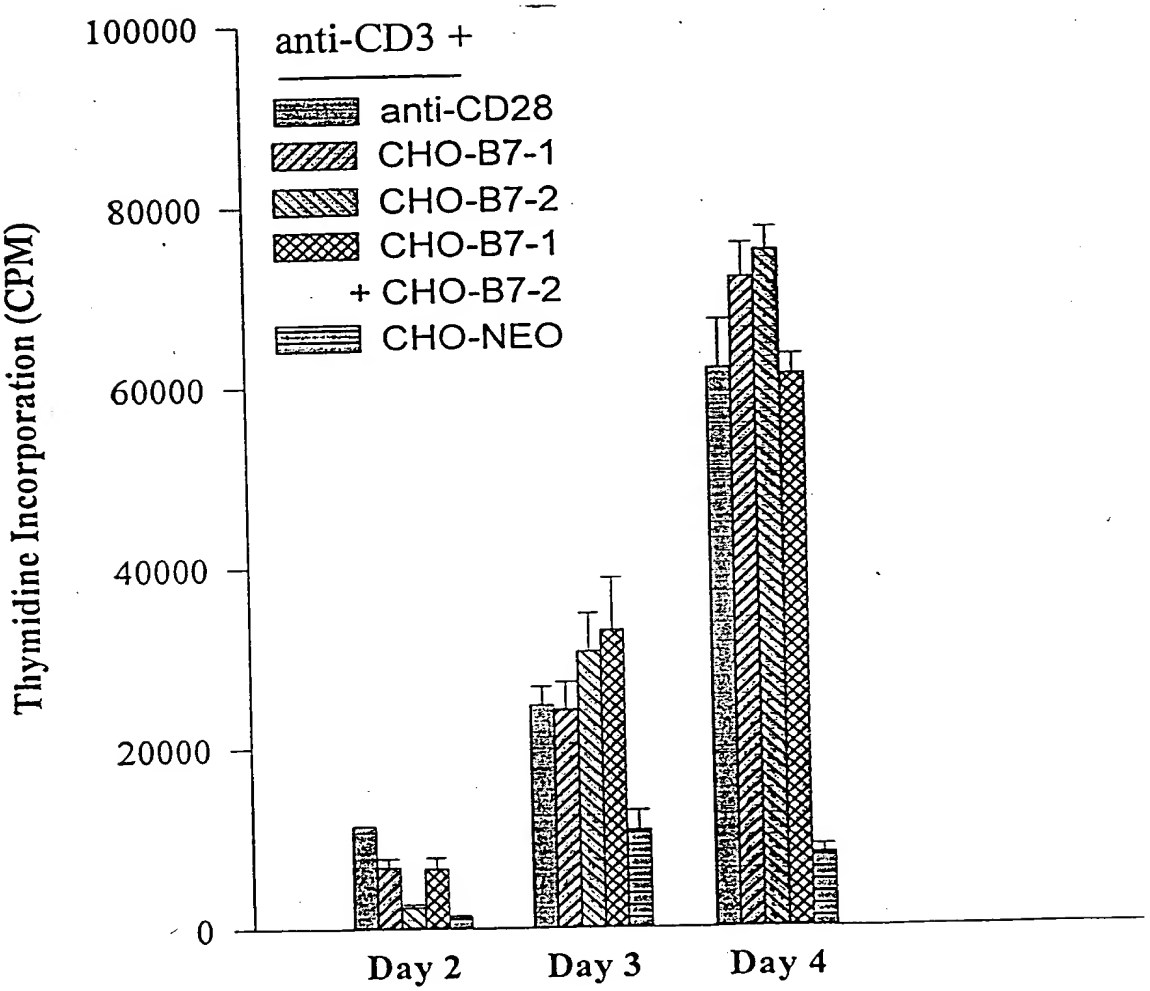

100000
80000
60000
40000
20000
0
Thymidine Incorporation (CPM)
anti-CD3 +
anti-CD28
CHO-B7-1
CHO-B7-2
CHO-B7-1
+ CHO-B7-2
CHO-NEO
Day 2
Day 3
Day 4

FIG. 19

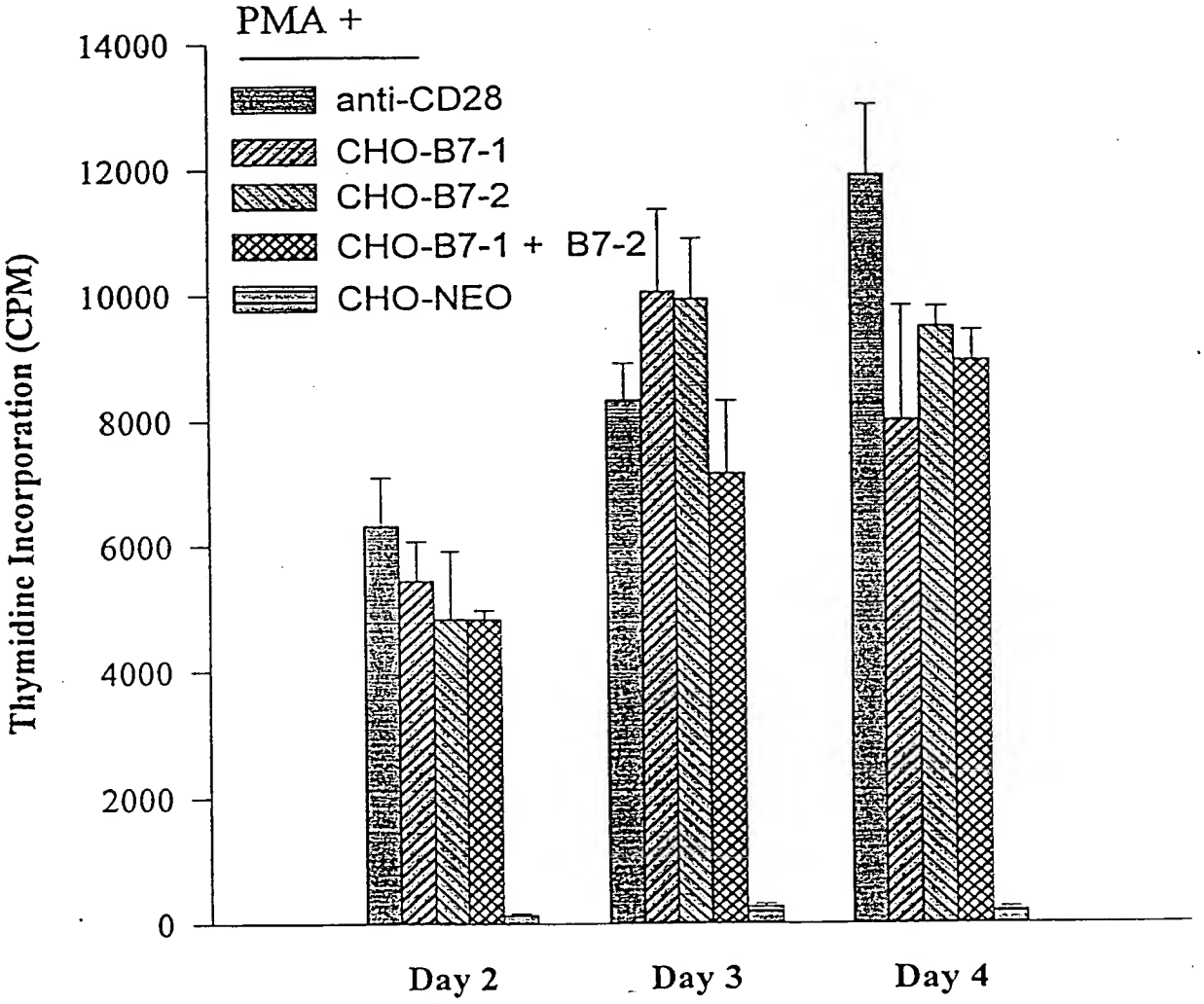

PMA +
anti-CD28
CHO-B7-1
CHO-B7-2
CHO-B7-1 + B7-2
CHO-NEO
Thymidine Incorporation (CPM)
14000
12000
10000
8000
6000
4000
2000
0
Day 2
Day 3
Day 4

FIG. 20

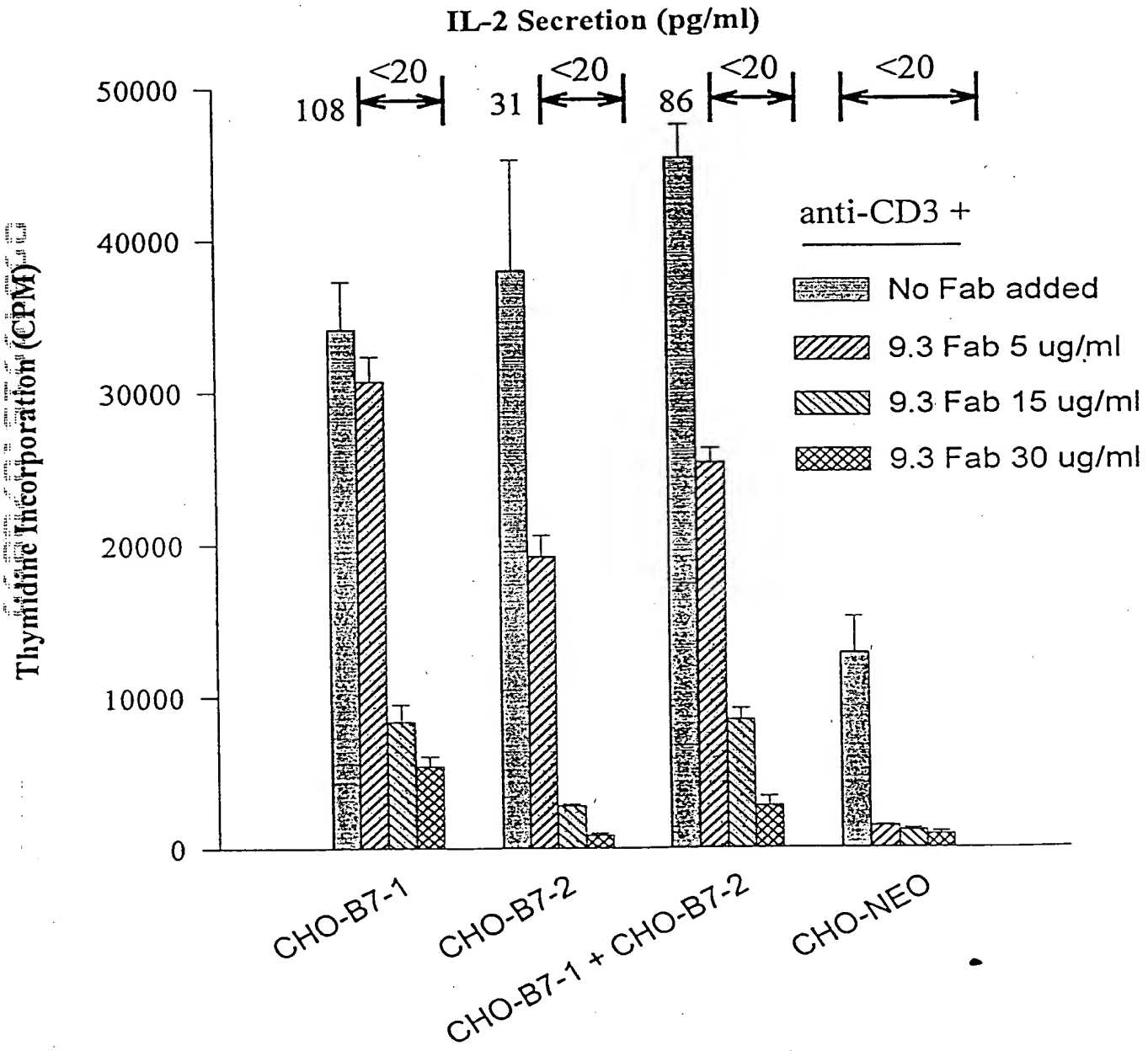

IL-2 Secretion (pg/ml)
108
<20
31
<20
86
<20
<20
50000
40000
30000
20000
10000
0
Thymidine Incorporation (CPM)
anti-CD3 +
No Fab added
9.3 Fab 5 ug/ml
9.3 Fab 15 ug/ml
9.3 Fab 30 ug/ml
CHO-B7-1
CHO-B7-2
CHO-B7-1 + CHO-B7-2
CHO-NEO

FIG. 21

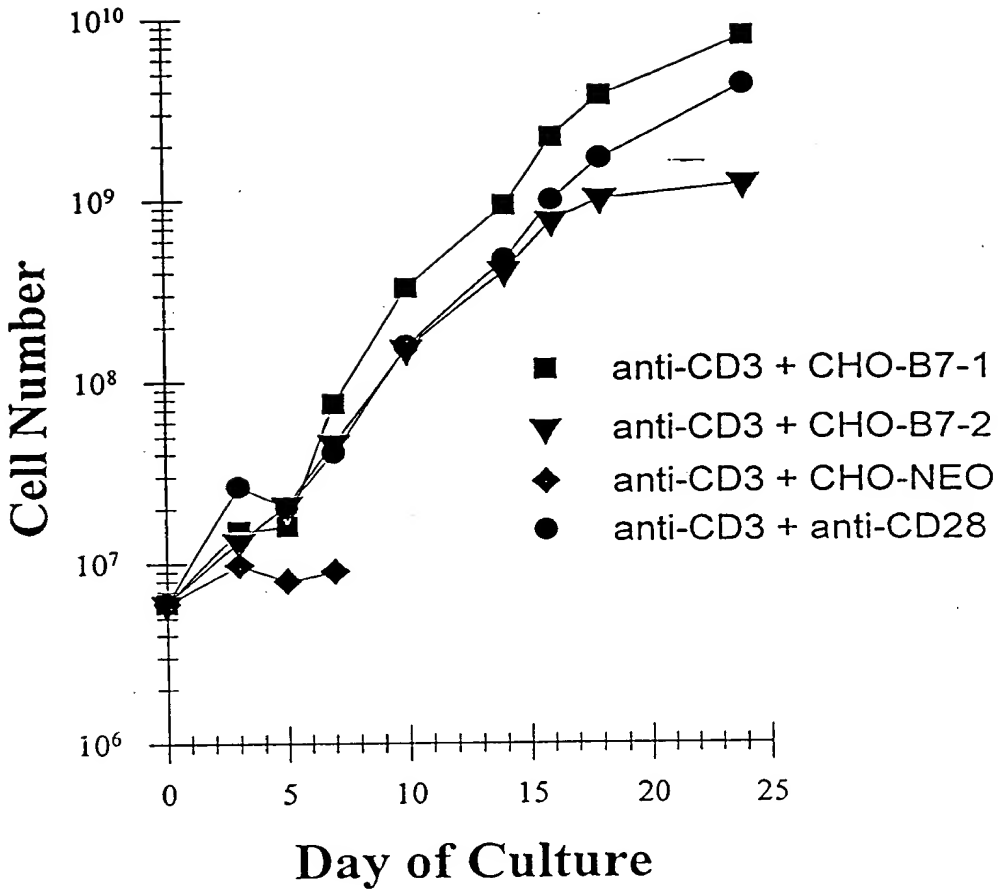

10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
Cell Number
0
5
10
15
20
25
Day of Culture
anti-CD3 + CHO-B7-1
anti-CD3 + CHO-B7-2
anti-CD3 + CHO-NEO
anti-CD3 + anti-CD28

FIG. 22

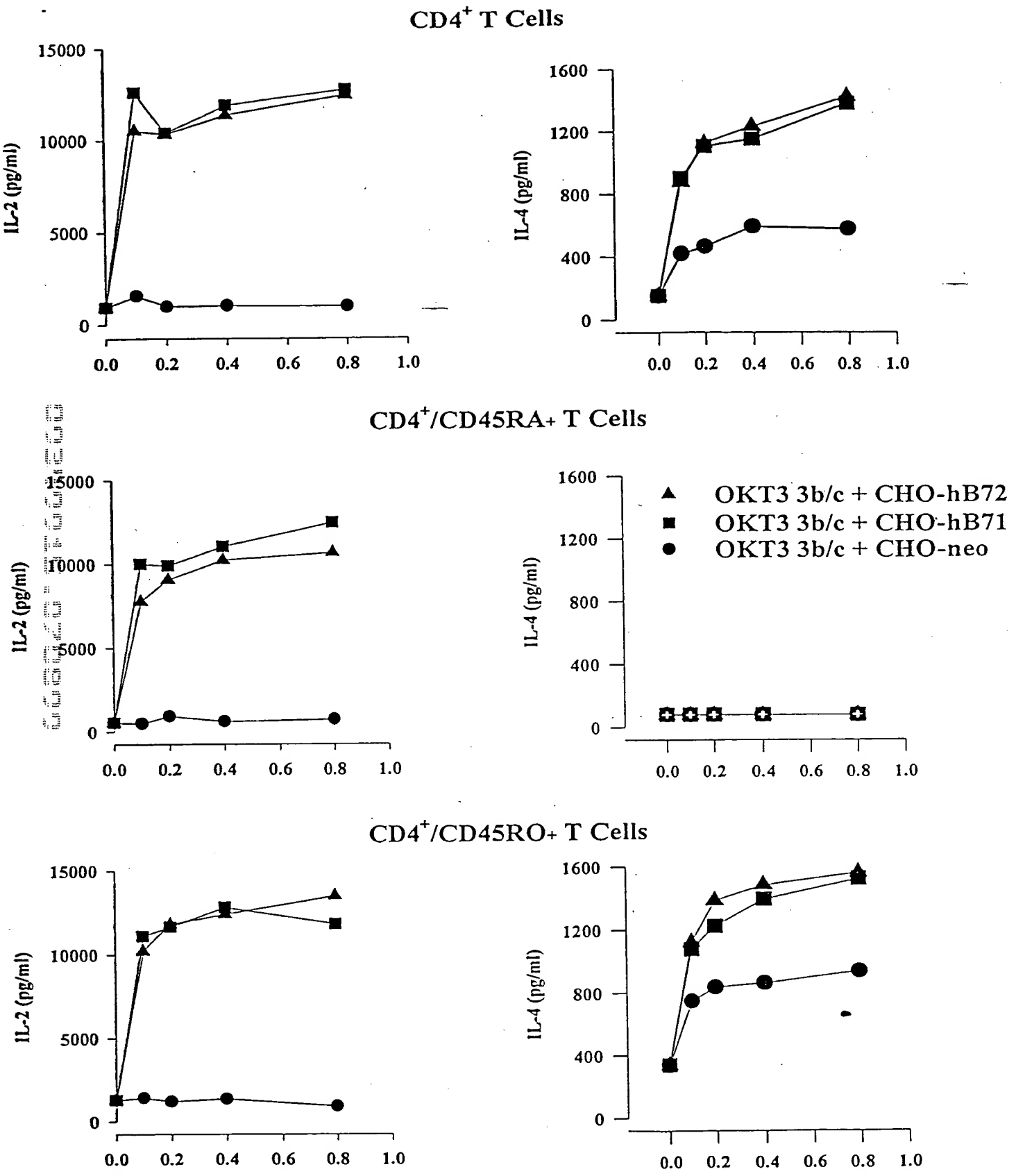

CD4+ T Cells
IL-2 (pg/ml)
15000
10000
5000
0
0.0
0.2
0.4
0.6
0.8
1.0
IL-4 (pg/ml)
1600
1200
800
400
0
CD4+/CD45RA+ T Cells
CD4+/CD45RO+ T Cells
OKT3 3b/c + CHO-hB72
OKT3 3b/c + CHO-hB71
OKT3 3b/c + CHO-neo
CHO Cells Per T Cell

FIG. 23

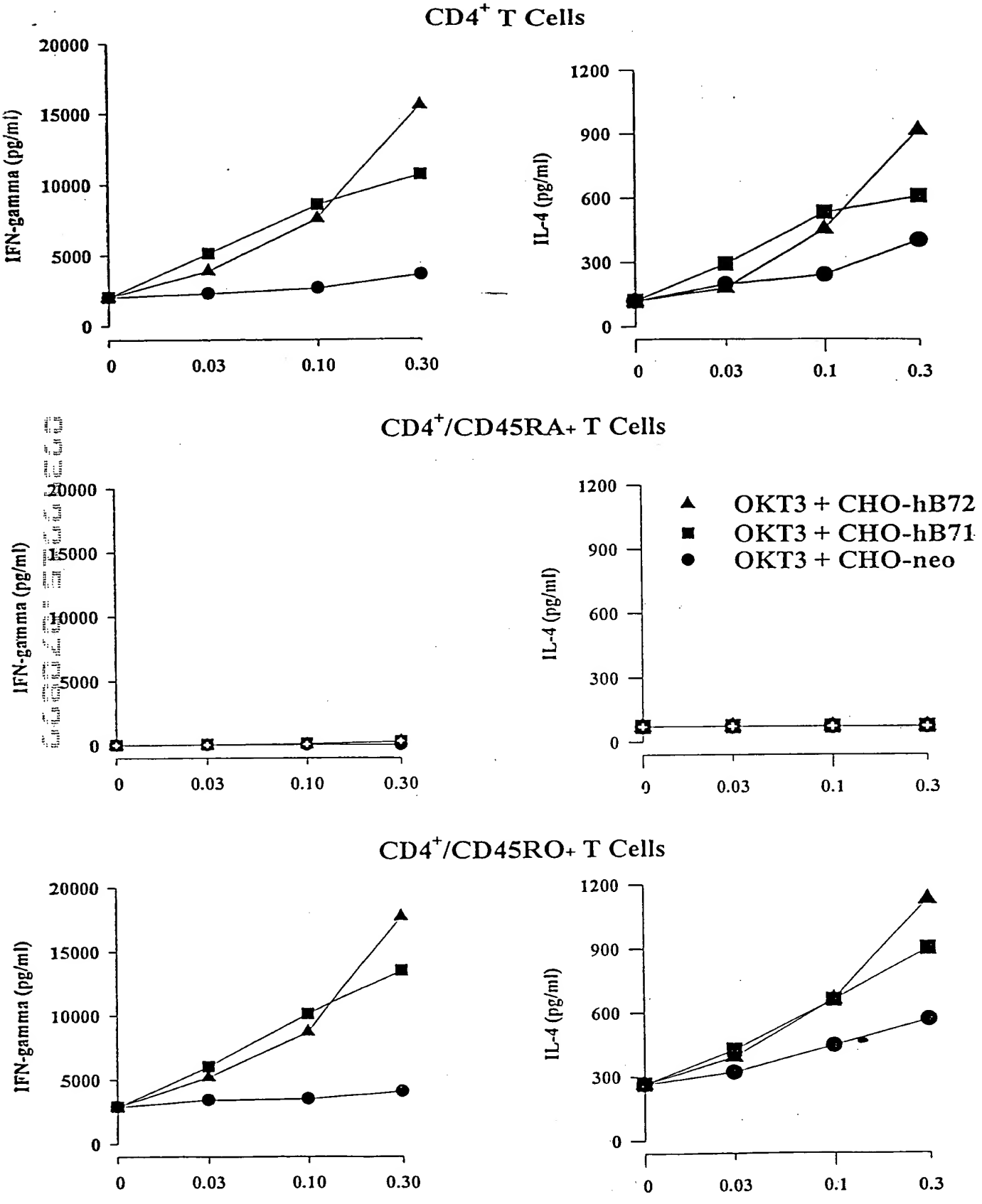
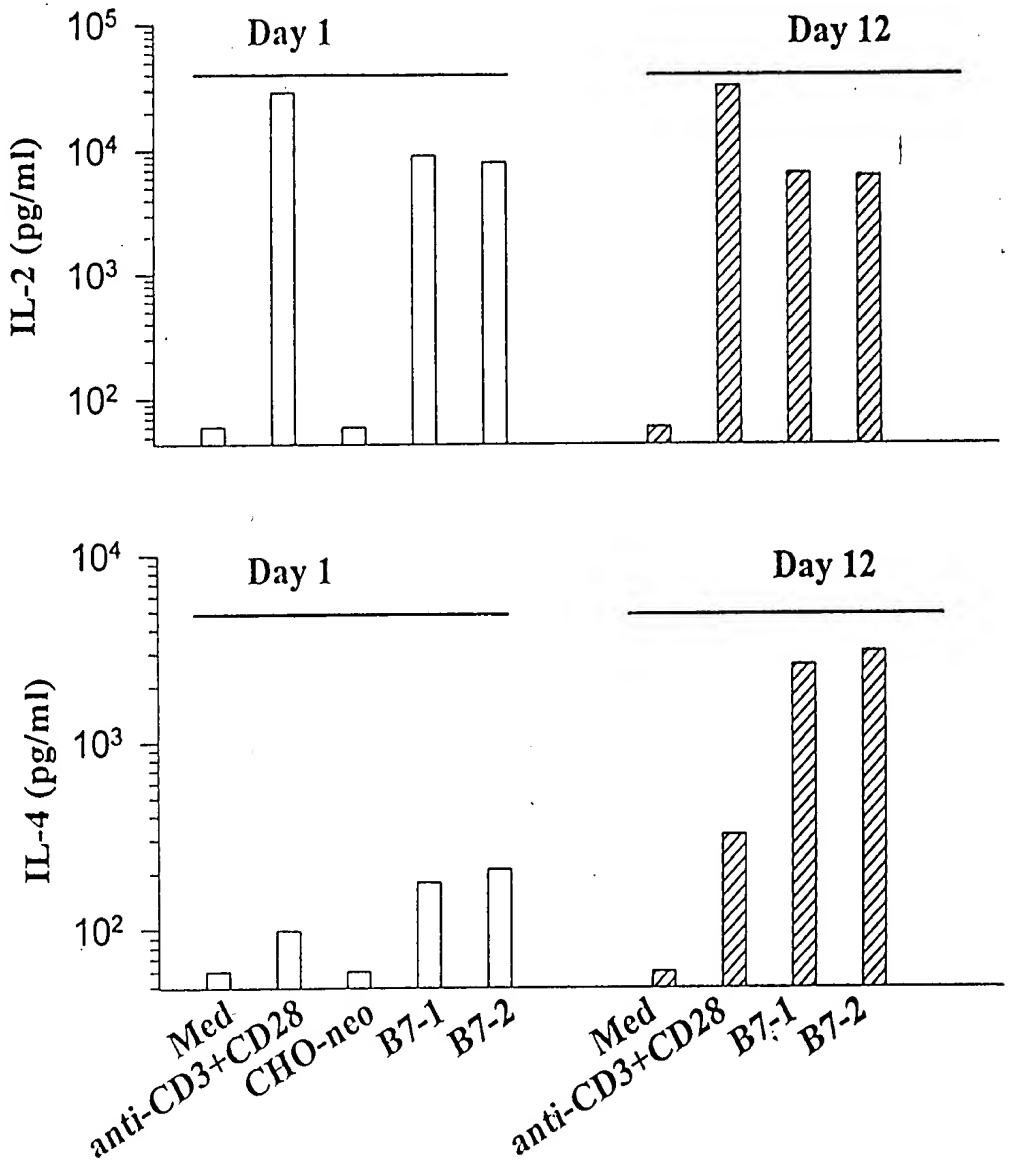

CD4+ T Cells
IFN-gamma (pg/ml)
20000
15000
10000
5000
0
0
0.03
0.10
0.30
IL-4 (pg/ml)
1200
900
600
300
0
0
0.03
0.1
0.3
CD4+/CD45RA+ T Cells
IFN-gamma (pg/ml)
IL-4 (pg/ml)
OKT3 + CHO-hB72
OKT3 + CHO-hB71
OKT3 + CHO-neo
CD4+/CD45RO+ T Cells
IFN-gamma (pg/ml)
IL-4 (pg/ml)
CHO Cells Per T Cell

FIG. 24

FIG. 3E



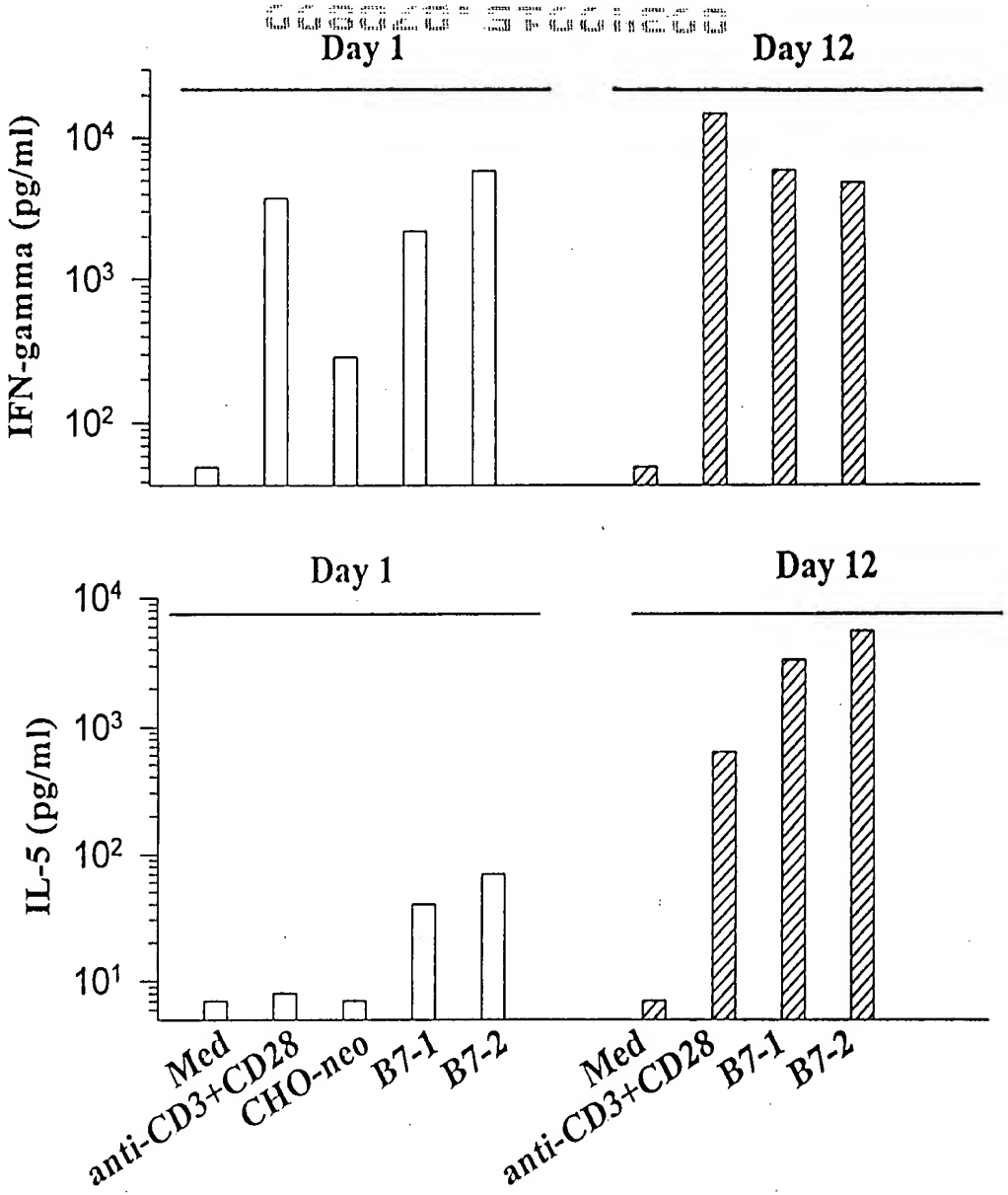

Day 1
Day 12
IFN-gamma (pg/ml)
10^4
10^3
10^2
Day 1
Day 12
IL-5 (pg/ml)
10^4
10^3
10^2
10^1
Med
anti-CD3+CD28
CHO-neo
B7-1
B7-2
Med
anti-CD3+CD28
B7-1
B7-2

Fig. 24

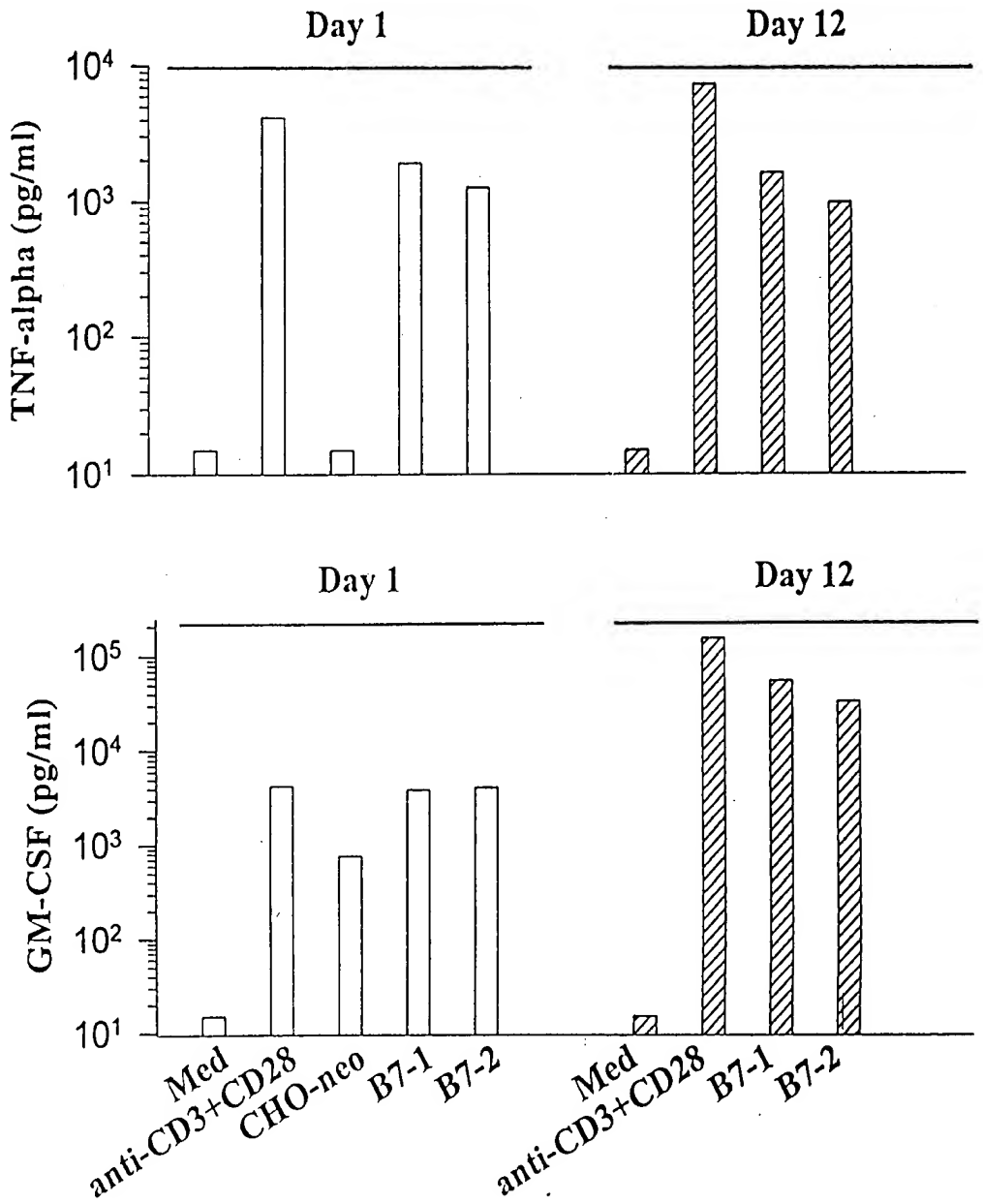


FIG. 24

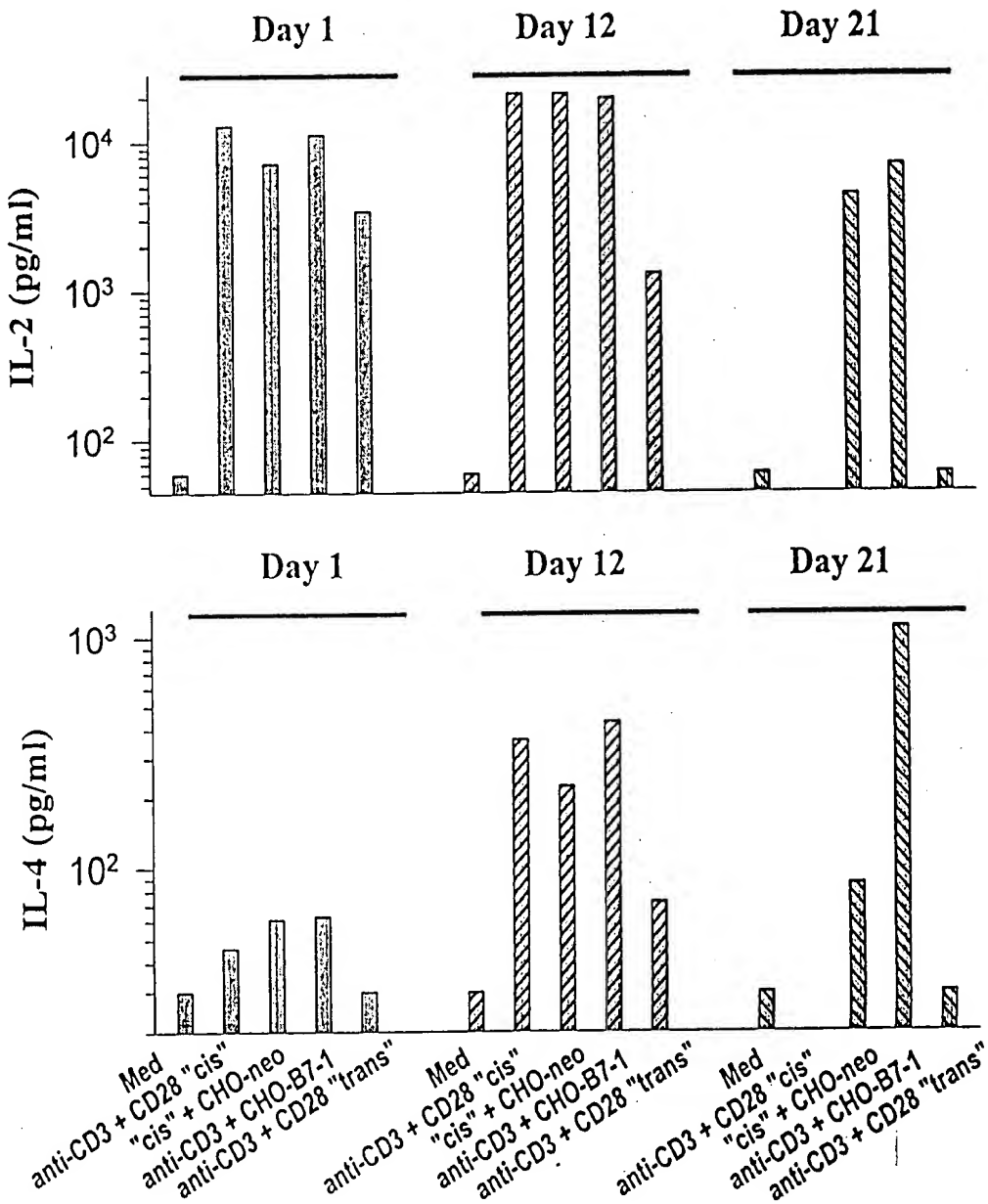


FIG. 2B

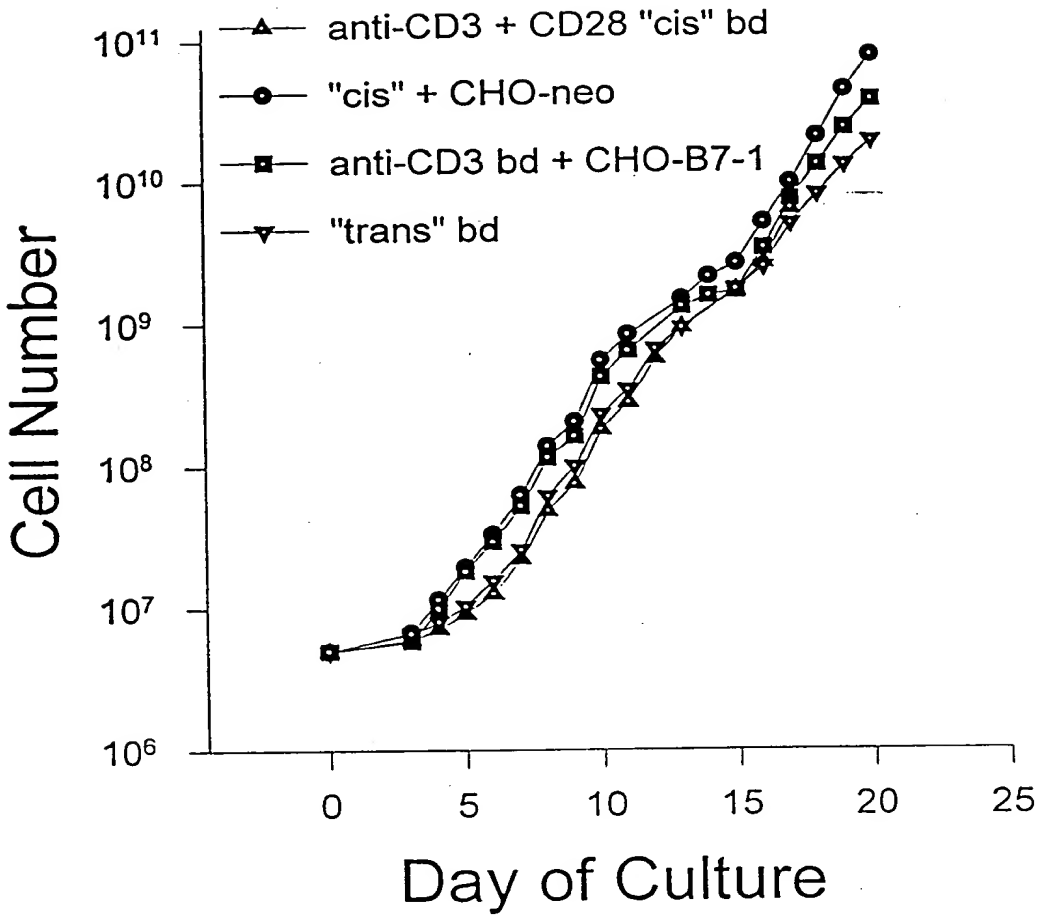

anti-CD3 + CD28 "cis" bd
"cis" + CHO-neo
anti-CD3 bd + CHO-B7-1
"trans" bd
Cell Number
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
0
5
10
15
20
25
Day of Culture

FIG. 29

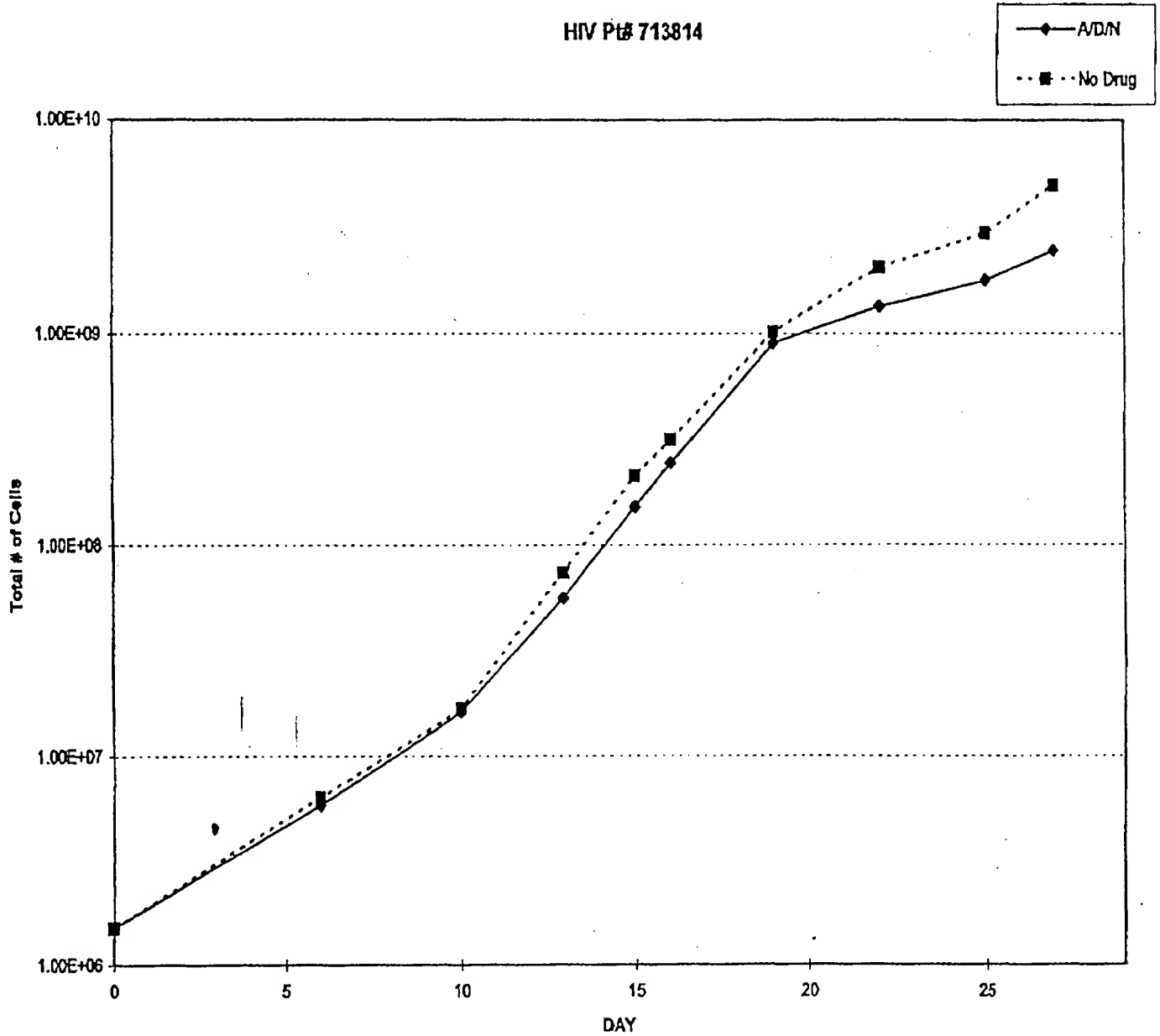

HIV Pt# 713814
A/D/N
No Drug
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
Total # of Cells
0
5
10
15
20
25
DAY

FIG. 30

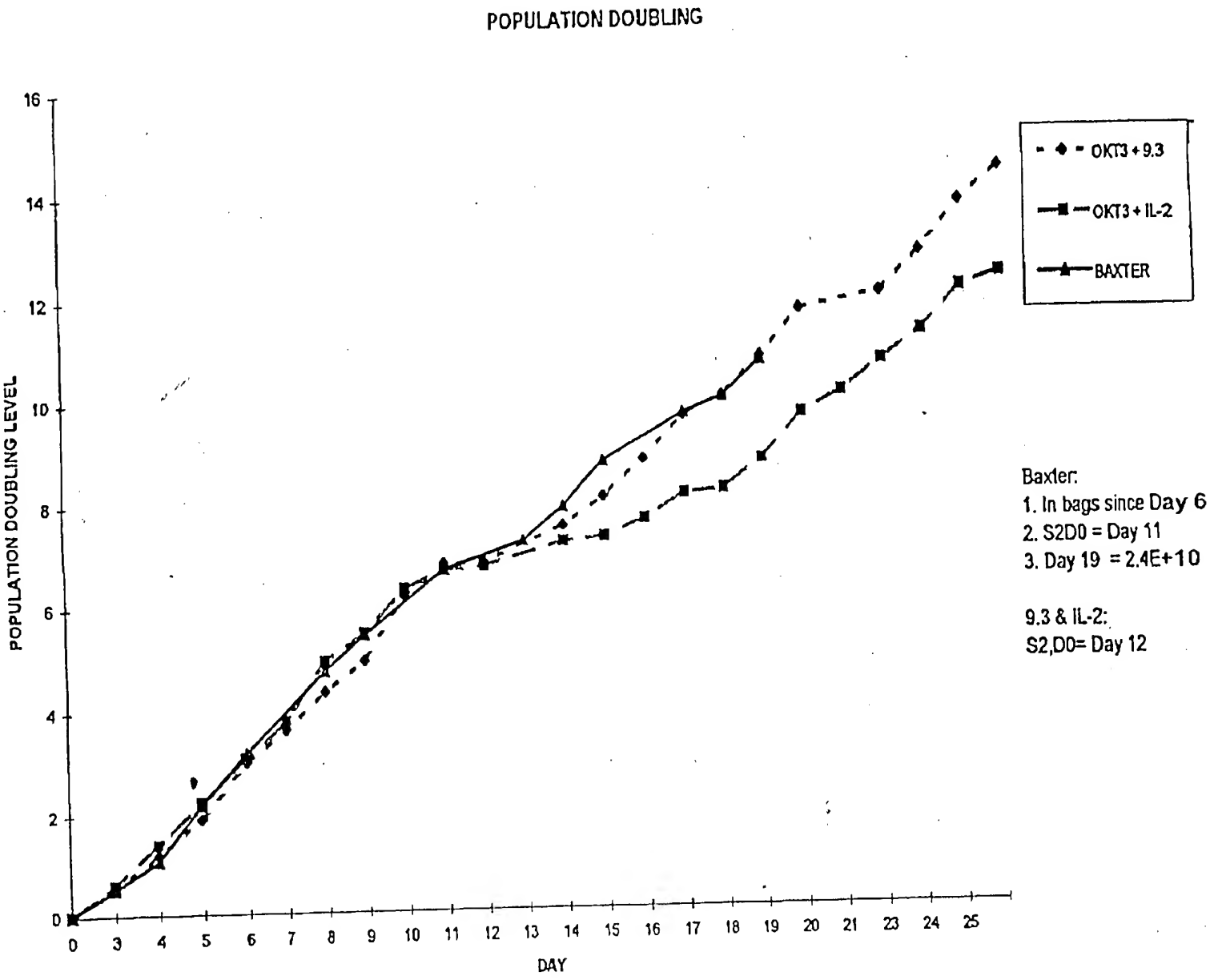


FIG. 31